Claims

1. A process to allow the characterization of a microbial gene or genes, here gene,

where said gene encodes a gene product;

where said gene product is a gene target;

where said gene target is important to a microbe's ability to infect or sustain an infection in a mammal, where said microbe is:

genetically altered to become a genetically altered microbe, such that the amount of said gene product produced by said genetically altered microbe is regulated and controlled by a Tetracycline-Controllable Element or TCE;

where said TCE is a gene regulatory system that controls the expression of the target gene or gene product, through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence;

where said gene, which may be any gene which encodes a microbial protein, or more generally a microbial gene product, is regulated by said TCE such that said gene produces either greater or lesser amounts of gene product, depending upon whether or not said genetically altered microbe is exposed to tetracycline;

where said mammal is a plurality of at least two or more mammals with said mammals are initially exposed to tetracycline and infected with said genetically altered microbe;

followed by:

the removal of the tetracycline exposed to a portion of said mammals, such that there is at least mammals one or one group of said mammals exposed to tetracycline and another one or group of not exposed to tetracycline;

followed by:

a comparison of the degree of infection, microbe levels, or physiological condition of the mammals exposed to tetracycline, compared to the degree of infection, microbe levels, or physiological condition of mammals not exposed to tetracycline;

followed by:

the identification of said genes, important to a microbe's ability to infect or sustain an infection in a mammal, where the comparison of the mammals exposed to tetracycline compared to the mammals not exposed to tetracycline shows a meaningful difference between the two groups of animals.

2. The process of claim 1, where said TCE is a gene regulatory system that controls the expression of the target gene or gene product, through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence, operably linked to a polynucleotide sequence encoding a reporter gene.

3. The process of claim 2, where said tetracycline-controllable transcription promoter polynucleotide sequence, is a prokaryotic transcription promoter.

4. The process of claim 1, where said TCE is a gene regulatory system that controls the expression of the target gene or gene product, through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable transcription promoter polynucleotide sequence, operably linked to a polynucleotide sequence encoding a reporter gene (RG) and a target gene (TG).

5. The process of claim 4, where said reporter gene is β-lactamase.

6. The process of claim 1, where said microbe has, in addition to the genetic alterations of claim 1, additional genetic alterations comprising a tetracycline resistance (or protection) and repressor DNA cassette (TRRDC).

7. The process of claim 6, where said TCE is a gene regulatory system that controls the expression of the target gene or gene product, through its ability to modulate the function of said gene in response to said microbe's exposure to tetracycline, and where said TCE is comprised of a tetracycline-controllable

transcription promoter polynucleotide sequence, operably linked to a polynucleotide sequence encoding a reporter gene and a target gene

and where the TCE, the TRRDC, the RG, and the TG are all on the same DNA cassette, which may be referred to as a Regulatory DNA Cassette or RDC.

8. The process of claim 6, where the TRRDC, comprises the structural gene *tetM*, the structural gene *tetR* and where a promoter is operably linked to the TCE.

9. The process of claim 1, where said meaningful difference between the two groups of animals is a mathematically significant difference in the survival rates or the levels of microbes, or levels of infection present in the mammals.

10. The process of claim 9, where said meaningful difference between the two groups of animals is a mathematically significant difference in the survival rates of the groups of animals.

11. The process of claim 10, where said significant difference in the survival rates of the groups of animals shows that animals exposed to tetracycline have poorer health, higher rates of infection, lower survival or higher levels of microbes than animals not exposed to tetracycline.

12. The process of claim 7, where the tetracycline resistent gene of said TRRDC is comprised of sequences from the *Staphylococcus aureus tetM* gene.

13. The process of claim 12, where said tetracycline repressor gene of said TRRDC is derived from the Tn10 transposon.

14. The process of claim 13, where said Tn10 transposon is selected from the sequence of SEQ. ID. NO. 35 and 36.

15. The process of claim 1 where said mammals are mice.

16. The process of claim 1, wherein said recombinant bacterium is a Staphylococcus species.

17. The process of claim 1, wherein said Staphylococcus species is *Staphylococcus aureus*.

18. The process of claim 1, wherein said microbe is a virus.

19. The process of claim 1, wherein said microbe is a lower eukaryote.

20. The process of claim 1, wherein said microbe is a yeast.

21. An isolated DNA molecule for integrating a heterologous polynucleotide sequence at a pre-determined location in a prokaryotic chromosome to operably control an endogenous prokaryotic gene, said DNA molecule comprising recombining element (RE) and a tetracycline controllable element (TCE), said TCE comprising a tetracycline-controllable prokaryotic transcription promoter polynucleotide sequence flanked at its 5' end by said RE, said RE comprising additional polynucleotide sequences of sufficient length for homologous recombination between the isolated DNA molecule and the prokaryotic chromosome.

22. The isolated DNA molecule of claim 21 further comprising a polynucleotide sequence encoding a reporter gene operably linked to said TCE.

23. The isolated DNA molecule of claim 22 wherein said reporter gene is beta-lactamase.

24. The isolated DNA molecule of claim 21 further comprising at least one prokaryotic transcription terminator polynucleotide sequence positioned between the RE and the TCE.

25. The isolated DNA molecule of claim 21 further comprising a polynucleotide sequence encoding a prokaryotic tetracycline resistance protein operably linked to a prokaryotic transcription promoter polynucleotide sequence positioned between the RE and the TCE.

26. The isolated DNA molecule of claim 25 wherein the tetracycline resistance protein is derived from the *Staphylococcus aureus tetM* gene.

27. The isolated DNA molecule of claim 21 further comprising a polynucleotide sequence encoding a prokaryotic tetracycline repressor protein operably linked to a tetracycline-controllable prokaryotic transcription promoter polynucleotide sequence positioned between the RE and the TCE.

28. The isolated DNA molecule of claim 27, wherein the tetracycline repressor is a a *tetR* gene derived from the Tn10 transposon.

29. A recombinant vector comprising the isolated DNA molecule of claim 21 in a form suitable for transformation of a host cell.

30. A host cell comprising the recombinant vector of claim 29.

31. A prokaryotic host cell comprising the DNA molecule of claim 21 wherein the DNA molecule is integrated at a pre-determined location in the host cell chromosome.

32. The isolated DNA molecule of claim 21, wherein said recombining elements are comprised of polynucleotides selected from Sequence ID NO: 40, 41, 42 and 43.

33. The isolated DNA molecule of claim 21, wherein said recombining elements are comprised of polynucleotides selected from Sequence ID NO: 44 and 45.

34. The isolated DNA molecule of claim 21, wherein said tetracycline-controllable element is comprised of polynucleotide Sequence ID NO: 37.

35. The isolated DNA molecule of claim 21, further comprising a polynucleotide sequence encoding a reporter gene operably linked to said tetracycline-controllable element.

36. The isolated DNA molecule of claim 21, wherein said tetracycline-controllable element is comprised of polynucleotide Sequence ID NO: 37.

37. The isolated DNA molecule of claim 21, wherein said reporter gene is beta-lactamase.

38. The isolated DNA molecule of claim 21, wherein said reporter gene is beta-lactamase, selected from SEQ ID NO: 38 and 39.

39. The isolated DNA molecule of claim 21 wherein the tetracycline resistance protein is derived from the *Staphylococcus aureus tetM* gene.

40. The isolated DNA molecule of claim 21 wherein the tetracycline resistance protein is derived from the *Staphylococcus aureus tetM* gene selected from SEQ ID NO: 34.

41. The isolated DNA molecule of claim 21 wherein the tetracycline repressor is a a *tetR* gene derived from the Tn10 transposon and selected from SEQ ID NO: 35 and 36.

42. The isolated DNA molecule of claim 21, further comprising a polynucleotide sequence comprising at least one prokaryotic transcription terminator seqeunce (SEQ ID NO:33) positioned between the tetracycline-controllable element and one recombining element.

43. The isolated DNA molecule of claim 21, further comprising a polynucleotide sequence encoding a prokaryotic tetracycline resistance protein operably linked to a transcription promoter polynucleotide sequence.

44. The isolated DNA molecule of claim 21, further comprising a polynucleotide sequence encoding a tetracycline repressor protein operably linked to a transcription promoter polynucleotide sequence.

45. A recombinant vector comprising the isolated DNA molecule of claim 21 in a form suitable for transformation of a host cell.

46. An isolated DNA molecule for integrating a polynucleotide sequence including tetracycline-controllable elements (TCE) at a pre-determined location in a target DNA molecule, said isolated DNA molecule comprising the following DNA elements fused in sequence:

a) a first prokaryotic transcription terminator polynucleotide sequence;

b) a second prokaryotic transcription terminator polynucleotide sequence;

c) a polynucleotide sequence encoding a prokaryotic tetracycline resistance protein;

d) a polynucleotide sequence encoding a prokaryotic repressor protein;

e) a first tetracycline-controllable prokaryotic transcription promoter polynucleotide sequence;

f) a second tetracycline-controllable prokaryotic transcription promoter polynucleotide sequence; and

g) a polynucleotide sequence encoding a reporter protein;

said isolated DNA molecule comprising a polynucleotide sequence including the TCE flanked at the end opposite the polynucleotide sequence encoding said reporter protein by additional polynucleotide sequences of sufficient length for homologous

recombination between the isolated DNA molecule and the target DNA molecule at a pre-determined location.

47. A recombinant vector comprising the isolated DNA molecule of claim 46 in a form suitable for transformation of a host cell.

48. A prokaryotic host cell comprising the DNA molecule of claim 46 wherein the DNA molecule is integrated at a pre-determined location in the host cell chromosome.

49. The isolated DNA molecule of claim 46, wherein said recombining elements are comprised of polynucleotides selected from Sequence ID NO: 40, 41, 42 and 43.

50. The isolated DNA molecule of claim 46, wherein said recombining elements are comprised of polynucleotides selected from Sequence ID NO: 44 and 45.

51. The isolated DNA molecule of claim 46, wherein said tetracycline-controllable element is comprised of polynucleotide Sequence ID NO: 37.

52. The isolated DNA molecule of claim 46, further comprising a polynucleotide sequence encoding a reporter gene operably linked to said tetracycline-controllable element.

53. The isolated DNA molecule of claim 46, wherein said tetracycline-controllable element is comprised of polynucleotide Sequence ID NO: 37.

54. The isolated DNA molecule of claim 46, wherein said reporter gene is beta-lactamase.

55. The isolated DNA molecule of claim 46, wherein said reporter gene is beta-lactamase, selected from SEQ ID NO: 38 and 39.

56. The isolated DNA molecule of claim 46, wherein the tetracycline repressor protein is derived from the *Staphylococcus aureus tetM* gene.

57. The isolated DNA molecule of claim 46, wherein the tetracycline repressor protein is derived from the *Staphylococcus aureus tetM* gene selected from SEQ ID NO: 34.

58. The isolated DNA molecule of claim 46, wherein the tetracycline repressor is a *tetR* gene derived from the Tn10 transposon and selected from SEQ ID NO: 35 and 36.

59. An isolated DNA molecule comprising a tetracycline-controllable transcription promoter polynucleotide sequence operably linked to a microbial gene.

60. The isolated DNA molecule of claim 59, wherein said tetracycline-controllable element is comprised of polynucleotide Sequence ID NO: 37.

61. The isolated DNA molecule of claim 60, further comprising a polynucleotide sequence encoding a reporter gene operably linked to said tetracycline-controllable element.

62. The isolated DNA molecule of claim 61, wherein said reporter gene is beta-lactamase.

63. The isolated DNA molecule of claim 62, wherein said reporter gene is beta-lactamase selected from SEQ ID NO: 38 and 39.

64. The isolated DNA molecule of claim 59, further comprising a polynucleotide sequence encoding a a tetracycline resistance (or protection) and repressor DNA cassette (TRRDC) operably linked to a transcription promoter polynucleotide sequence.

65. The isolated DNA molecule of claim 64, further comprising a polynucleotide sequence encoding a prokaryotic a tetracycline resistance (or protection) and repressor DNA cassette (TRRDC), operably linked to a transcription promoter polynucleotide sequence.

66. The isolated DNA molecule of claim 65, wherein said tetracycline resistance (or protection) and repressor DNA cassette (TRRDC) is derived from a *Staphylococcus aureus tetM* gene.

67. The isolated DNA molecule of claim 66, wherein said tetracycline resistance (or protection) and repressor DNA cassette (TRRDC) is derived from the *Staphylococcus aureus tetM* gene comprised of SEQ ID NO: 34.

68. The isolated DNA molecule of claim 67, wherein said tetracycline resistance (or protection) and repressor DNA cassette (TRRDC) is a TN10 derived tetracycline repressor.

69. The isolated DNA molecule of claim 68, wherein the a tetracycline resistance (or protection) and repressor DNA cassette (TRRDC) is a TN10 derived tetracycline repressor selected from the polynucleotides of SEQ ID NO: 35 and 36.

70. A recombinant vector comprising the isolated DNA molecule of claim 21 in a form suitable for transformation of a host cell.

71. A host cell comprising the recombinant vector of claim 70.

72. A recombinant vector comprising the isolated DNA molecule of claim 46 in a form suitable for transformation of a host cell.

73. A host cell comprising the recombinant vector of claim 72

74. A recombinant vector comprising the isolated DNA molecule of claim 59 in a form suitable for transformation of a host cell.

75. A host cell comprising the recombinant vector of claim 74.

76. A process to regulate expression of an endogenous prokaryotic gene comprising the cultivation of the prokaryotic cell in medium with a controlled amount of tetracycline or a tetracycline analog.

77. A process to regulate expression of an endogenous prokaryotic gene comprising the cultivation of the prokaryotic cell in a mammalian host with a controlled amount of tetracycline or a tetracycline analog.

sub B2

Add B3